The Incidence of Caseous Lymphadenitis in Alberta Sheep and Assessment of Impact by Vaccination with Commercial and Experimental Vaccines

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ABSTRACT

In Alberta, caseous lymphadenitis (CLA) is one of the leading causes of lamb and mutton carcass condemnation. In this study, serologic results confirmed a high (50–94%) incidence of exposure to *Corynebacterium pseudotuberculosis*, the causative agent of CLA, in mature, unvaccinated sheep in southern Alberta. To assess the efficacy and impact of vaccination with 2 commercial (Glanvac-6 and Case-Vac) and 1 experimental (WC+MDP−GDP) CLA vaccines, a series of 3 field trials in 3249 ewes and lambs was conducted in affected flocks from 1992–1996. Efficacy was assessed from the serological response to vaccination, prevalence and size of injection site reactions by treatment, and the incidence of CLA abscesses. Overall, agglutinating antibody titres to *C. pseudotuberculosis* in lambs vaccinated with WC+MDP−GDP and Case-Vac remained significantly elevated above nonvaccinated control lambs for the 12 mo period after the initial vaccination. Lambs vaccinated with the WC+MDP−GDP maintained higher titres ($P < 0.06$) than those vaccinated with Case-Vac for the period from 6 to 12 mo after vaccination. Agglutinating antibody titres for lambs vaccinated with Glanvac did not differ from those of controls at any point during the 12 mo period after vaccination. The number of injection site reactions was elevated in lambs vaccinated with Glanvac as compared to those vaccinated with WC+MDP−GDP but the size of injection site reactions did not significantly differ. Sheep vaccinated with WC+MDP−GDP also had a reduced incidence of putative CLA abscesses, although confirmation of the presence of *C. pseudotuberculosis* was only successful in a small number of instances.

RÉSUMÉ

La lymphadénite caséeuse est une des causes majeures de rejet des carcasses de mouton ou d'agneau en d'Alberta. Les résultats sérologiques présentés ici, confirment que dans le sud de cette province il existe, chez les ovins adultes non vaccinés, une trés forte incidence (50–94 %) de contamination par *Corynebacterium pseudotuberculosis*, agent causal de la lymphadénite. Pour déterminer l'efficacité et l'impact d'une vaccination, trois essais de terrain incluant 3249 brebis ou agneaux ont été effectués entre 1992 et 1996 dans des troupeaux où la lymphadénite sévit à l'état endémique. En 1992, l'efficacité de Glanvac-G (Vetrepharm Inc., London, Ontario) a été comparée avec celle d'un vaccin expérimental contenant des germes entiers tués associés au muramyl dipeptide-sn-glyceryl-dipalmitoyl (WC+MDP−GDP, National Animal Disease Center, Ames, Iowa, USA). Dans cette étude 620 agneaux du troupeau du centre de recherche de Lethbridge ont été utilisés. En 1993, l'efficacité de Case Vac (Colorado Serum Company, Denver, Colorado, USA) a été comparée à celle du vaccin WC+MDP−GDP chez 453 agneaux du même troupeau. De 1994 à 1996 l'efficacité du vaccin WC+MDP−GDP a été testée à nouveau chez 2 176 agneaux ou brebis appartenant à neuf troupeaux commerciaux dans le sud de l'Alberta. Tous les animaux ont reçu 2 vaccinations à quatre semaines d'intervalle. L'efficacité a été évaluée par la réponse humorale à la vaccination, le nombre et la taille des abcès au site d'injection et l'incidence d'abcès imputables à la lymphadénite. En résumé des anticorps agglutinants anti-*C. pseudotuberculosis* sont apparus un mois après le rappel chez les agneaux vaccinés par le vaccin WC+ MDP−GDP ou par Case-Vac et leurs titres sont restés significativement élevés par rapport à ceux des animaux témoins pendant les douze mois suivant la première immunisation. Les agneaux vaccinés avec le vaccin WC+MDP−GDP ont maintenu des titres plus élevés que les animaux ayant reçu Case Vac entre le sixème et le douzième mois suivant le début du traitement. Les titres d'anticorps agglutinants des agneaux ayant reçu Glanvac n'ont jamais été différents de ceux des témoins pendant les douze mois suivant le début de la vaccination. Le nombre d'abcès au site d'injection a été plus élevé dans le groupe Glanvac que dans le groupe vaccin WC+MDP−GDP, mais la taille des abcès formés n'était pas significativement différentes entre les différents groupes y compris le groupe témoin et le groupe Case-Vac. Les animaux

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This study was supported by the Alberta Agricultural Research Institute, LRC contribution #3879697.

Received January 6, 1997.

vaccinés avec le vaccin WC+MDP−GDP ont également développé moins d'abcès imputables à la lymphadénite bien que la confirmation de la présence de germes n'ait pu être obtenue que dans un petit nombre de cas.

(Traduit par les auteurs)

INTRODUCTION

Caseous lymphadenitis (CLA) is a common disease of sheep caused by the bacterium, *Corynebacterium pseudotuberculosis*. The disease is characterized by abscesses formed in both the peripheral lymph nodes and visceral organs (1–4). CLA is of major economic consequence to sheep producers in many parts of the world, including North America (1,3) and Australia (5,6). In Alberta, CLA is one of the leading causes of lamb and mutton carcass condemnation. Furthermore, trimming CLA lesions increases labor at the abattoir and decreases saleable meat yield. Between 3–5% of mutton and 0.02–0.03% of federally inspected lamb carcasses in Alberta are condemned due to CLA, with another 8% of all carcasses trimmed to remove CLA lesions. Other industry costs include reduced wool production (6), reduced sales of breeding stock (4), reduced reproductive efficiency (7), and increased rates of culling and mortality of breeding stock (1,3).

Once CLA is endemic, the disease is almost impossible to eradicate (2). Antibiotic therapy is generally not effective as antimicrobials are unable to penetrate the heavily encapsulated lesions. Control programs usually entail culling animals with recurring abscesses, isolation of affected animals, and lancing of abscesses. Identification of infected animals is often difficult as internal abscesses, especially those in the lungs, escape detection (8) while external abscesses are difficult to detect in sheep carrying a full fleece (3). Use of an effective vaccine may be the most promising method of controlling this disease. Currently only one *C. pseudotuberculosis* vaccine (Glanvac) is commercially available in Canada. Another vaccine, containing inactivated whole cells of *C. pseudotuberculosis* and muramyl dipeptide (MDP), has been shown to protect lambs against CLA in laboratory and field trials (9), but is not commercially available (10).

The Lethbridge Research Centre flock has a high incidence of CLA (approximately 25% of mature sheep are seropositive) and we wanted to determine the proportion of animals seropositive to *C. pseudotuberculosis* in this flock and other southern Alberta sheep flocks. Second, we wanted to assess the efficacy of an experimental whole cell vaccine containing the synthetic adjuvant muramyl dipeptide-sn-glyceryl-dipalmitoyl (WC+MDP−GDP) and compare the efficacy to Glanvac-6, and Case-Vac. MDP is the minimal structure essential for the adjuvant effect of mycobacteria in Freund's complete adjuvant and may be better than previously used adjuvants for potentiating convential whole cell vaccines. MDP-sn-glyceryl-dipalmitoyl is an improved analog of MDP untested as an adjuvant on a large scale.

MATERIALS AND METHODS

ANIMALS

In 1992, 620 lambs of Rambouillet, Suffolk, Finn, Dorset and Romanov breeding (born at the Lethbridge Research Centre in March and April) were used. In 1993, 453 lambs born in March and April (Rambouillet, Suffolk, Dorset) or September and October (Romanov and Finn) were used. In 1994–1996, 9 flocks containing 2176 ewes and lambs in Southern Alberta (8 commercial flocks less than 100 km from the Lethbridge Research Centre and the Lethbridge Research Centre flock) were used. CLA was present in all flocks, but the disease was not recognized as a major problem by any of the flock owners. Three flocks had previously participated in a CLA control program including annual vaccination, while ewes in other flocks had not previously received CLA vaccine. Owners of flocks on CLA control programs were instructed to withhold CLA vaccine from their flock for at least 10 mo prior to the study. All animals were handled according to guidelines established by the Canadian Council on Animal Care.

SEROLOGICAL TESTING

Blood was collected by jugular venipuncture, placed on ice, and transported to the Lethbridge Research Centre. Blood was centrifuged at 3000 × *g* at 4°C for 30 min. Serum was collected and stored at −40°C. Serum samples were collected prior to vaccination from all animals (mature stock and lambs) and 1, 3, 6, 9, and 12 mo after vaccination. Agglutinating antibody titres (11) were determined, in duplicate, at the National Animal Disease Center. The microagglutination test detects antibody to cell-associated antigens but does not detect antibody to *C. pseudotuberculosis* exotoxin. The microagglutination test was used because it is rapid, easy to use on large numbers of samples, and can distinguish among vaccinated and nonvaccinated animals.

VACCINES

Glanvac-6 (Vetrepharm Inc., London, Ontario) and Case-Vac (Colorado Serum Co., Denver, Colorado, USA) were used. An untested vaccine, WC+MDP−GDP, was prepared at the National Animal Disease Center. Briefly, *C. pseudotuberculosis* strain ATCC 19410 was grown, inactivated, and dried as previously described (9,10). MDP−GDP (2-acetamido-2-deoxy-3-O-D-2-propionyl-L-alanyl-D-isoglutamine-D-glucopyranose-sn-glyceryl-dipalmitoyl) was synthesized. To prepare the vaccine, inactivated bacterial cells (1.17 mg/mL) and MDP−GDP (1.25 mg/mL) were prepared in 0.14 M NaCl containing 0.2% sorbitan-mono-oleate and 0.1% formalin. Bacterial cells (0.86 mL/dose) and MDP-GDP (0.4 mL/dose) were added to light mineral oil and emulsified. Final concentrations were 1.0 mg bacterial cells and 50 μg MDP-GDP in 10% oil (per mL dose).

IMMUNIZATION

Sheep and lambs with microagglutination titres > 1:320 to *C. pseudotuberculosis* after the initial serologic testing were not vaccinated. A 1:320 cutoff was used in this study for the following reasons. The mean titre for all Lethbridge Research Station lambs prior to vaccination was 1:168 with a standard deviation of 529. Although titres up to 1:10 240 were found in lambs from natural exposure, the

TABLE I. Prevaccination agglutinating antibody titres to *Corynebacterium pseudotuberculosis* in 9 flocks (n = 2176) in southern Alberta 1994–1996

Flock	Titre of lambs (%)				Titre of sheep (%)			
	No.	< 80[a]	160–320[b]	> 320[c]	No.	< 80	160–320	> 320
1	75	82.7	16.0	1.3	198	41.9	37.9	20.2
2	130	80.8	19.2	0.0	155	8.4	42.6	49.0
3	213	92.0	6.6	1.4	277	13.7	58.8	27.4
4	179	77.1	22.9	0.0	219	27.8	43.8	28.3
5[d]	53	92.4	7.6	0.0	48	22.9	64.6	12.5
6	6	83.3	16.7	0.0	18	50.0	16.7	33.3
7	88	88.6	10.2	1.2	64	6.2	56.2	37.5
8[d]	114	97.4	2.6	0.0	81	7.4	17.2	65.4
9[d]	258	90.3	8.5	1.2	0	0.0	0.0	0.0

[a] titres < 80. Unexposed to *C. pseudotuberculosis*
[b] titres 160–320. Questionable exposure to *C. pseudotuberculosis*
[c] titres > 320. Exposed to *C. pseudotuberculosis*
[d] Flocks participating in CLA control program prior to study

majority of lambs had titres in the range of 1:40 to 1:80 and titres greater than 1:640 were uncommon. As the titre data were not normally distributed and had such a large standard error, sheep and lambs with titres greater than 1 standard deviation from the mean (i.e. 640 or greater) were excluded and considered as infected animals.

Three separate trials were performed to compare the serologic response, side effects of vaccination, and efficacy of Glanvac, Case-Vac, and WC+MDP–GDP. In 1992, lambs were assigned to one of 3 groups: control (no vaccine), WC+MDP–GDP, or Glanvac. In 1993, lambs were randomly assigned to one of 3 groups: control (no vaccine), WC+MDP–GDP, or Case-Vac). In 1994–1996, ewes and lambs were assigned to 2 groups: control (saline) or WC+MDP–GDP.

All animals were inspected for external abscesses prior to vaccination and were randomly assigned into treatment groups. Lambs were a minimum of 8 wk of age (average 10 wk of age) prior to the first vaccination. All injections were made high on the neck; this site was chosen for ease of both administration of vaccine and determination of presence and severity of injection-site reactions. The WC+MDP–GDP was administered intramuscularly. Saline, Glanvac, and Case-Vac vaccines were administered subcutaneously. This methodology is in accordance with label instructions for Glanvac, but is counter to those for Case-Vac which specify injection in the axillary space. Case-Vac was not injected in the axillary space due to reports of lameness and subsequent reductions in lamb performance when injections are made at this site (12). All animals received a booster injection of the appropriate vaccine 4 wk after the initial inoculation.

ASSESSMENT OF INJECTION SITE REACTIONS

The injection site was palpated at shearing. Areas of localized swelling, firmness, unusual thickness, or the presence of nodular lumps was noted and subjectively classified as small (< 5 mm, containing residual scar tissue), medium (5–10 mm), or large (> 10 mm) lesions.

DETERMINATION OF CLA INFECTION

Lambs were housed with CLA infected sheep in contaminated facilities. External abscesses, when noted, were lanced, and purulent material was collected on a sterile swab. Swabs were then submitted to the Alberta Agriculture Food and Rural Development Animal Health Laboratory in Lethbridge. Duplicate samples were plated on trypticase soy agar with 5% defibrinated sheep blood and incubated either aerobically or in 5% CO_2 at 35°C for 24-to-72 h. *C. pseudotuberculosis* was confirmed from the presence of hemolysis and positive catalase and urease reactions (13).

STATISTICAL ANALYSES

For each of the years 1992–1994, analysis of variance (14) were carried out on the antibody titre data from the Lethbridge Research Centre animals. Analysis were performed on titre data obtained at each of the blood sampling dates up to 12 mo to assess the effect of breed, sex, vaccine treatment (control, WC+MDP–GDP, Glandvac, and Case-Vac) and interactions among these factors. Data for animals with antibody titres (> 1:320 in the initial serum sample) were excluded from the statistical analysis due to the high likelihood of prior infection with *C. pseudotuberculosis*. Also, data from samples taken more than 12 mo after the initial vaccination were not used since most of the animals had been slaughtered by then. A $\log_2$ (X/10) transformation was applied to the observed titre (X) data to stabilize the variances. Repeated measures analyses of variance were carried out over the months to determine if the titre response over the 12 mo period was consistent for the breeds, vaccine treatments and sexes. Wilk's lambda statistic was used for significant tests involving the factor months.

Similar analysis were carried out on the titre data from industry flocks in 1994–1996 with effects due to flock, sex (ewe, ram or wether), treatment (control or WC+MDP–GDP), sampling time and interactions among these factors being included in the statistical model. The analyses were carried out for mature or lamb age groups separately since not all flocks had all ages of sheep, and previous analysis indicated that there was a significant ($P < 0.001$) effect of age on antibody titre.

Categorical data analysis techniques (15) were used to study the association between vaccine treatment and the prevalence and size of injection site reactions in lambs and ewes. The statistical analysis above were carried out using the General Linear Models (GLM) and Categorical Modelling (CATMOD) procedures of SAS (16).

RESULTS

SEROLOGY

50–94% of the Lethbridge Research Centre Flock had antibodies to *C. pseudotuberculosis* prior to initial vaccination (Table I). Log titres prior to initial vaccination were 0.26 (0.04 SEM) for 1992, 2.67 (0.07 SEM) for 1993, and 1.67 (0.10 SEM) for 1994. There were no significant differences ($P > 0.05$) in titres among treatment groups for each of these years before the initial vaccination (data not

shown). The repeated measures analysis of variance indicated that there were highly significant ($P < 0.001$) vaccine treatment × sampling time (month) interactions in each year which indicated that the development of the titres over the 12 mo period was not consistent for the vaccine treatments (Fig. 1–3). Significant ($P < 0.05$) interactions of breed and sex with month were evident in one or more of the years, but the nature of the interactions are not reported here since the effect of vaccine treatment accounted for most of the variation and the other effects were inconsistent and of minor interest in this study. Prior to the booster, titres were significantly ($P < 0.001$) higher than the controls for the Case-Vac group in 1993 and the WC+MDP−GDP group in all years of the study and remained higher ($P < 0.001$) than the controls to month 12. However, lambs vaccinated with Glandvac did not develop significantly higher ($P > 0.05$) titres than the controls in 1992, and titres of the Case-Vac group and WC+MDP−GDP were similar over the 12 mo period in 1993.

In the industry flock vaccine field trial (1994 to 1996), there were no significant differences ($P > 0.05$) in titres among treatment groups before the initial vaccination (data not shown). In all industry flocks, antibody titres for both lambs and mature stock were significantly higher than those of controls immediately prior to, and 1 mo after, the booster vaccination (Fig 4). Significant ($P < 0.001$) interactions of age were evident. In flocks 1, 5, and 6, attrition of lambs was severe by the 12-mo sample, while changes in flock 2 and 8 management precluded collection of samples 12 mo after initial vaccination. Adequate numbers of all age and treatment groups were only available in flocks 3, 4, and 7 to allow determination of antibody titres 12 mo after the initial vaccination. In flock 3, titres of vaccinated ewes and lambs remained higher than those of controls ($P < 0.001$) 12 mo after vaccination (Fig. 5). For flocks 4 and 7, at the same time point, titres of vaccinated lambs were markedly higher than those of controls ($P < 0.001$), although the difference between control and vaccinated ewes was less pronounced ($P < 0.05$).

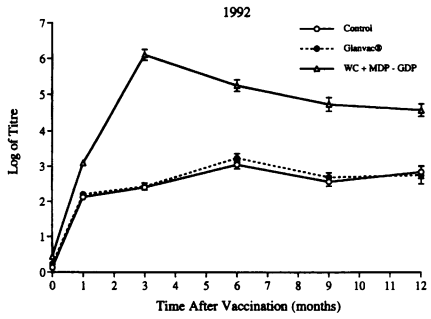

Figure 1. Titres of sheep in the Lethbridge Research Centre Flock vaccinated with Glanvac and WC+MDP−GDP, 1992.

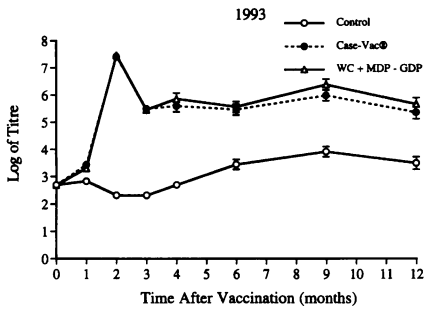

Figure 2. Titres of sheep in the Lethbridge Research Centre Flock vaccinated with Case-Vac and WC+MDP−GDP, 1993.

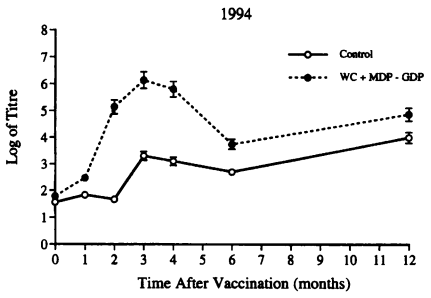

Figure 3. Titres of sheep in the Lethbridge Research Centre Flock vaccinated with WC+MDP−GDP, 1994.

PRESENCE AND SEVERITY OF INJECTION SITE REACTIONS

At the Lethbridge Research Centre, injection-site reactions were significantly more numerous ($P < 0.001$) in sheep vaccinated with Glanvac than in sheep vaccinated with WC+MDP−GDP (Table II). However, the size of injection site reactions did not differ between WC+MDP−GDP and Glanvac vaccinated groups ($P > 0.05$).

In lambs from industry flocks, the numbers and sizes of injection site reactions did not differ ($P > 0.05$) between control and WC+MDP−GDP groups (Table III). Results from 1992 and 1994 demonstrated that the

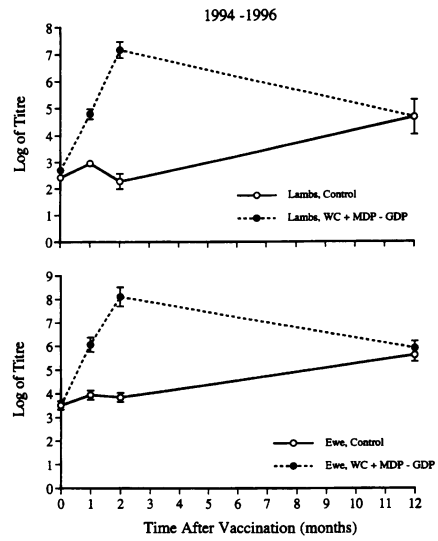

Figure 4. Titres of ewes and lambs from industry flocks vaccinated with WC+MDP−GDP, 1994.

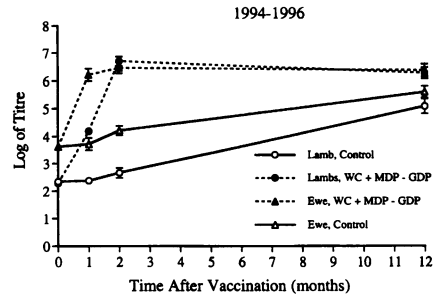

Figure 5. Titres of ewes and lambs from industry flocks with no previous CLA control; industry flock 3, 1994–1996.

number and severity of WC+MDP−GDP injection site reactions were not increased either in the short (3 wk post-booster) or long-term (4 to 10 mo post-booster) compared to those associated with injection of saline or Glanvac.

PRESENCE OF PUTATIVE CLA ABSCESSES

The number of CLA-like abscesses (other than at the injection site reactions) were significantly reduced ($P < 0.001$) in sheep vaccinated with WC+MDP−GDP compared to control sheep (Table III). Overall, abscesses from 51 sheep were lanced and exudate cultured. However, no sheep with titres prior to vaccination of less than 1:640 were positively cultured for presence of *C. pseudotuberculosis*. Positive cultures were identified in a small ($n = 5$) number of mature ewes with pre-vaccination titres ≥ 1:640. The majority of

TABLE II. Prevalence and size of injection site reactions 3 wk post-booster vaccination of the Lethbridge Research Centre Flock in 1992

Reactions	WC+MDP−GDP	Glanvac vaccine	Significance[b]
Number[a]			
0	201	152	$P < 0.001$
1	11	56	
2	0	14	
Size			
< 5.0 mm	8	53	$P > 0.05$
5.0–10.0 mm	1	14	
> 10.0 mm	2	3	

[a] Maximum number of reactions = 2, at each site of initial vaccination and booster
[b] Effect of treatment (WC+MDP−GDP vs. Glanvac vaccine)

TABLE III. Prevalence of injection-site reactions and other abscesses at shearing (4–10 mo post-booster vaccination) from 1994–1996

Abscess	Treatment	Lamb (< 1 y)	Ewe[a] (> 1 y)
Injection site reactions			
None	WC+MDP−GDP	144	291
	CONTROL	104	183
Small (< 5 mm)	WC+MDP−GDP	0	7
	CONTROL	0	3
Large (> 10 mm)	WC+MDP−GDP	0	0
	CONTROL	0	4
Other abscesses			
None	WC+MDP−GDP	137	177
	CONTROL	90	77
Present	WC+MDP−GDP	7	121
	CONTROL	14	113

[a] sheep with initial titres < 1:640

putative CLA abscesses were found to contain *Staphylococcus* spp. and *Actinomyces pyogenes*.

DISCUSSION

Corynebacterium pseudotuberculosis is an insidious organism that is difficult to control once established in flocks. The majority of sheep in the Lethbridge Research Centre flock, not previously vaccinated for CLA, had a high agglutinating antibody titre (50 to 94%), suggesting exposure to *C. pseudotuberculosis*. Similarly, mature sheep and lambs from industry flocks, without previous CLA control programs, also had a high incidence of exposure to *C. pseudotuberculosis*; 54 to 94% and 11.4 to 22.9%, respectively. Lambs from industry flocks with previous CLA control programs had a lower incidence ($P < 0.01$) of exposure to *C. pseudotuberculosis* (range 2.6 to 9.7%).

Use of an effective vaccine may be the most promising method of controlling CLA. Vaccines containing inactivated *C. pseudotuberculosis* cells (9,10,17,18) or *C. pseudotuberculosis* toxoids (19,20) have been developed but their effectiveness is questionable. Two of the 3 vaccines used in this study induced high agglutinating antibody titres. Most lambs vaccinated with WC+MDP−GDP and Case-Vac developed titres > 1:320 within 1 mo after the booster vaccination similar to earlier work (9,10,17,18). However, some lambs did not (1.89 to 7.66%) and remained negative for the 12-mo period.

When titres were compared at each sampling time up to and including 12 mo after initial vaccination, those of lambs vaccinated with WC+MDP−GDP did not differ significantly ($P > 0.15$) from those of lambs treated with Case-Vac. However, in repeated measures analysis, titres of lambs vaccinated with WC+MDP−GDP were higher ($P < 0.06$) than those of lambs vaccinated with Case-Vac from 6 to 12 mo after vaccination.

Significant ($P < 0.05$) effects of breed and sex, treatment by breed, and treatment by sex were also noted in all years of the study. However, these breed and sex differences were not consistent over all years. As immune response to vaccination is mediated by numerous factors including age of lamb, antibody titres of dams to *C. pseudotuberculosis* (21), and other stressors including disease (22), it is possible that breed or sex variation in any of these factors could have resulted in significant breed and sex effects.

One vaccine, Glanvac, did not induce agglutinating antibody titres. Between 22 and 32% of control and 24% of Glanvac lambs had negative antibody titres for a 12-mo period. Glanvac is a toxoid based vaccine and would not expect to induce agglutinating antibody to cell-associated antigens. However, the inability of this vaccine to induce antibodies measured by other tests has been described by others (21). In that work, lambs vaccinated at 8 and 12 wk of age with Glanvac, provided their dams had high antibody titres, showed minimal reduction in infection with CLA compared to unvaccinated controls.

Side effects due to vaccination has always been of concern and differences were seen among the 3 vaccines. The potency of MDP−GDP allows lower doses of whole cells to be used without compromising protection, thus reducing the incidence of injection site reactions.

CLA is characterized by abscess formation in both the lymph nodes and visceral organs (1–4). Although serological data indicated that CLA was prevalent in Alberta sheep, throughout the study period, the flocks did not develop external abscesses making the efficacy assessment of these vaccines difficult. Few external abscesses were detected. Identification of infected animals who have internal abscesses, especially those in the lungs, are difficult to detect (8). Whether there was a change in the incidence of internal abscesses (e.g. pulmonary abscesses) among groups is not known.

Overall, Case-Vac and WC+MDP−GDP induced high microagglutinating antibody titres in vaccinated sheep. Sutherland et al (20) has shown that the serological response to vaccination is correlated with protection against CLA. Similarly, agglutinating titres correlate with a decrease in naturally occuring external abscesses (10). Maintainance of agglutinating titre over time should improve the protection of sheep against CLA, provided that animals receive booster vaccinations on an annual basis.

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